

# **Demonstration Evaluation of Biodegradable Degreaser**

## **Appendix D.**

**SMI Results from Eagle Kleen III Analytical Testing**

## **Appendix E.**

**SMI Results from Eagle Kleen III Vapor Pressure Testing**

## **Appendix F.**

**SMI Results from Eagle Kleen III Toxicity Testing**

**National Risk Management Research Laboratory  
Office of Research and Development  
U.S. Environmental Protection Agency  
Cincinnati, OH 45268**

## **Appendix D**

### **SMI Results from Eagle Kleen III Analytical Testing**

# SMI, Inc.

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Attn: Sara Kuczek  
Battelle Memorial Institute  
505 King Ave  
Columbus, OH 43201

Date: 07-Mar-2005

SMI/REF: 04DEC086  
*Final Report*

Product: **EAGLE KLEEN III** (Lot C531-84-1)  
(received 01-Dec-2004)

Dilution: As received

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*Modified testing (product was tested "as received" [undiluted] )*

**MIL-PRF-87937D** (24 Sep 2001)

**CLEANING COMPOUND, AEROSPACE EQUIPMENT**

**Type IV - Heavy Duty, Water Dilutable Cleaning Compound**

3.3	Toxicity	Informational
3.3.4	Biodegradability	Conforms
3.4	Compositional assurance	Informational
3.5	Chemical properties	
3.5.1	Chemical requirements	
	Insoluble matter	Conforms
	Flash point	Conforms
	Emulsion characteristics	Conforms*
	Wet adhesion tape test	Conforms*
	% Cleaning efficiency	Conforms*
	Terpene hydrocarbons	Not applicable
3.5.2	Residue rinsibility	Conforms*
3.6	Physical properties	
3.6.1	Heat stability	Does not conform*
3.6.2	Cold stability	Conforms
3.6.3	Rheology	
3.6.3.1	Consistency	Not applicable
3.6.3.2	Sprayability	Not applicable

\* Test performed using "as received" solution (ready to use) instead of dilution required by specification. Results should not be considered for QPL listing.

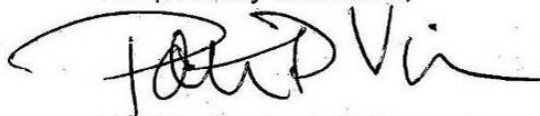
Client: Battelle Memorial Institute  
Product: **EAGLE KLEEN III Lot # C531-84-1**  
Dilution: Per specification  
MIL-PRF-87937D (Type IV)

Date: 07-Mar-2005  
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3.7	Effect on metals	
3.7.1	Hydrogen embrittlement	<u>Conforms*</u>
3.7.2	Total immersion corrosion	<u>Conforms*</u>
3.7.3	Low-embrittling cadmium plate corrosion	<u>Conforms*</u>
3.7.4	Effects on unpainted metal surfaces	<u>Conforms*</u>
3.7.5	Sandwich corrosion	<u>Conforms*</u>
3.7.6	Wet adhesion tape test	<u>Conforms*</u>
3.8	Effect on painted surfaces	<u>Conforms*</u>
3.9	Stress crazing of MIL-PRF-5425 and MIL-PRF-25690 (Type A and C) acrylic plastics	<u>Does not conform*</u>
3.10	Stress crazing of polycarbonate plastic	<u>Does not conform*</u>
3.11	Long-term storage stability	<u>Not performed</u>
3.12	Hot dip galvanizing corrosion	<u>Conforms*</u>
3.13	Workmanship	<u>To be Cert. by Mfr.</u>
3.14	Effect on polysulfide sealants	<u>Conforms*</u>
3.15	Rubber compatibility	<u>Conforms*</u>
3.16	Effect on polyimide insulated wire	<u>Conforms</u>

\* Test performed using "as received" solution (ready to use) instead of dilution required by specification. Results should not be considered for QPL listing.

Respectfully submitted,



Patricia D. Viani, SMI Inc.

Client: Battelle Memorial Institute  
Product: **EAGLE KLEEN III Lot # C531-84-1**  
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MIL-PRF-87937D (Type IV)

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3.1.1 Qualification (Initial): The cleaning compound furnished under this specification shall be a product which has been tested and has passed the qualification tests specified herein and has been listed or approved for listing on the applicable Qualified Products List (QPL).

3.3 Toxicity: The cleaning compound shall have no adverse effect on the health of personnel or the environment when used for its intended purpose and with proper personal protective equipment (when required). The product shall be evaluated for aquatic toxicity with a 96-hour Fathead minnow (*Pimephales promelas*) bioassay and a 48-hour *Ceriodaphnia dubia* bioassay in accordance with Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, EPA/600/4-90/027. The percent survival at 1, 10, 50, and 100 ppm shall be reported for both organisms.

% of Fathead Minnows ( <i>Pimephales promelas</i> ) Surviving			
Concentration	After 24 hours	After 48 hours	After 96 hours
1 ppm	100%	100%	100%
10 ppm	0%	0%	0%
50 ppm	0%	0%	0%
100 ppm	0%	0%	0%

% of Cladoceran ( <i>Ceriodaphnia dubia</i> ) Surviving		
Concentration	After 24 hours	After 48 hours
1 ppm	90%	50%
10 ppm	50%	20%
50 ppm	0%	0%
100 ppm	0%	0%

Result Informational

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- 3.3.4 Biodegradability: The supplier of the cleaning compound shall furnish certification from the surfactant manufacturers that the surfactants are readily biodegradable in accordance with 40 CFR, Part 796, Subpart D. Biodegradability testing shall be accomplished as specified in paragraph 4.5.22 on the finished product by an independent laboratory approved by the qualifying activity. Biodegradability on the finished product shall be determined over 28 days by the Shake Flask Method monitored by analysis of Total Organic Carbon (TOC). The Type I compound shall meet the requirement of a minimum of 75% biodegradable and Types II, III, and IV compounds shall meet the requirement of a minimum of 85 % biodegradable at the end of the 28-day period.

***Biodegradability after 28 days: 87.1 %***

Result Conforms

- 3.4 Compositional assurance: The cleaning compound shall be tested for nonvolatile matter as specified in paragraph 4.5.1. The concentrated cleaning compound and a 10% solution of the cleaning compound in distilled water shall be tested for pH as specified in paragraph 4.5.3. Results of these tests as well as an infrared spectrogram of the nonvolatile matter (see 4.8.2) and a gas chromatogram (see 4.8.1 for Type I only) shall be recorded by the qualifying activity for use in conformance inspections (see 4.3). Conformance inspection results for nonvolatile matter shall not differ by more than 2 percent absolute from the recorded value. Conformance inspection results for pH shall not differ by more than 1 pH unit from the recorded value. Conformance inspection infrared spectrograms and gas chromatograms shall show no significant difference when compared to the original qualifying spectrogram.

PROPERTY	RESULT
Nonvolatile matter	13.1 %
pH (undiluted)	6.9
pH (10%)	N/A (RTU)
Infrared spectrogram	Attached

Result Informational

- 3.5 Chemical properties.

- 3.5.1 Chemical requirements: The cleaning compound shall meet the requirements listed in Table I.

Client: Battelle Memorial Institute  
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### 3.5.1 Chemical requirements (continued):

TABLE I

Requirement	Type IV		Test Method
	Min.	Max.	
Insoluble Matter (WT%)	—	0.05	4.5.2
Flash Point (°F) 10 % solution concentrate	None <sup>1</sup> None <sup>1</sup>	— ---	4.5.7
Emulsion Characteristics (mls free water) 5 minutes 8 hours 24 hours	— — 11.0	5.0 — —	4.5.8
Wet Adhesion Tape Test	Pass		4.5.27
% Cleaning Efficiency	90 %	---	4.5.21
Terpene Hydrocarbons (% WT)	—	None	4.5.23

<sup>1/</sup> No flash point should be observed up to the boiling point of the compound.

4.5.2 Insoluble matter The percent insolubles shall be calculated as follows:

$$I = \frac{A-B}{W} \times 100$$

Where:

A = Final filter paper weight  
B = Initial filter paper weight  
W = Weight of sample  
I = % wt. insoluble matter

**Insoluble matter = < 0.01 %**

Result Conforms

4.5.7 Flash point: The flash point of the concentrated cleaning compound (Type I, II, III and IV) shall be determined in accordance with ASTM D 56 (Tag Closed Cup) and for materials that have a tendency to form a surface film under the test conditions, use ASTM D 93. The flash point of the 10% solution in distilled water (Type I only) shall be determined in accordance with ASTM D 92.

**No flash point observed to initial boiling point (212°F).**

Result Conforms

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### 3.5.1 Chemical requirements (continued):

- 4.5.8 Emulsion characteristics: Twenty ml of a 25% by volume solution (Types I and II) of the cleaning compound (12.5% by volume solution for Types III and IV) shall be placed in a 50 ml glass stoppered graduated cylinder. Twenty ml of lubricating oil conforming to MIL-PRF-2104, grade 10W, shall be added. An emulsion shall be formed by 10 inversions of the graduated cylinder followed by a vigorous 15-second shake. After the emulsion has stood for 5 minutes, the 15-second shake shall be repeated. At 5 minutes and 8 hours for the Type I and at 5 minutes and 24 hours for the Types II, III and IV cleaners, the amount of free water and cleaner which separates from the lubricating oil shall conform to the requirements of Table I.

Amount of free water remaining:

After 5 minutes fewer than 5 mls

After 24 hours: >11 mls

Result Conforms\*

- 4.5.21 Cleaning Efficiency: The cleaning efficiency of the cleaning compound shall be reported as the average of three test results and shall conform to the requirements of Table I.

Cleaning Efficiency: 98.5%

Result Conforms\*

- 4.5.23 Terpene hydrocarbons (Type I only): An approved test procedure shall be used.

Result Not applicable

- 3.5.2 Residue Rinsibility: When a freshly prepared solution of the cleaning compound is tested in accordance with 4.5.4, it shall not leave any residue or stains. A freshly prepared solution is defined as one being prepared no longer than 30 minutes prior to testing. The weight change shall be not greater than that obtained with standard hard water tested under the same conditions.

No residue nor stains; no weight change

Result Conforms\*

- 3.6 Physical properties (All types unless otherwise noted).

- 3.6.1 Heat stability: The concentrated cleaning compound, when tested in accordance with 4.5.5, shall show no marked color change or precipitation and shall not corrode or stain the AMS 5046 (SAE 1020) steel strip (a slight darkening of the steel strip shall not be objectionable). Layering or separation shall constitute failure if it does not return to its original homogeneous state upon cooling.

Visible corrosion of steel strip; rust-colored precipitation; no layering

Result Does not conform

\* Test performed using "as received" solution (ready to use) instead of dilution required by specification.



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- 3.6.2 Cold stability: The concentrated cleaning compound shall return to its original homogeneous condition when tested in accordance with 4.5.6.

**Compound returned to original homogeneous condition after 5 cycles**

Result Conforms

- 3.6.3 Rheology (Type III only).

- 3.6.3.1 Consistency: When tested as specified in 4.5.24, the concentrated cleaning compound shall flow between 10 and 20 centimeters in 10 seconds. The product shall also exhibit rheology, which enables it to meet the sprayability requirement.

Result Not applicable

- 3.6.3.2 Sprayability: The concentrated cleaning compound, when dispensed at 45 psig and tested in accordance with 4.5.25, shall give satisfactory spray characteristics and deposit a uniform layer on a vertical surface 3 feet away from the nozzle.

Result Not applicable

- 3.7 Effect on metals (All types unless otherwise noted).

- 3.7.1 Hydrogen embrittlement: When tested in accordance with 4.5.9, the concentrated cleaner (all types) and a 10% solution of the cleaner (Types I, II, and IV only) in distilled water shall not cause hydrogen embrittlement of cadmium plated or IVD aluminum coated AISI 4340 steel.

Specimens: Type 1c, *cadmium plated in accordance with Treatment B of ASTM F519*

**As received: No failures within 150 hours.**

**Dilute (10 %): Not performed**

Result Conforms\*

Specimens: Type 1c, *grit blasted, IVD Aluminum plated per MIL-DTL-83488D, Cl 2, Ty I.*

**As received: No failures within 150 hours.**

**Dilute (10 %): Not performed**

Result Conforms\*

\* Test performed using "as received" solution (ready to use) instead of dilution required by specification.

Client: Battelle Memorial Institute  
 Product: **EAGLE KLEEN III Lot # C531-84-1**  
 Dilution: Per specification  
 MIL-PRF-87937D (Type IV)

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- 3.7.2 **Total immersion corrosion:** When tested in accordance with 4.5.10 (ASTM F 483), the concentrated cleaning compound (all types) and a 10% solution of the cleaning compound (Types I, II and IV only) in distilled water shall not show any indication of staining, etching, pitting, or localized attack on any of the panels, or cause a weight change of an average of three (3) test panels greater than that shown in Table II. A slight discoloration of the panels shall not be objectionable. The cleaning compound shall not layer or separate for the duration of the test.

Table II Total Immersion Corrosion Requirements

Alloy	Weight Loss (mg/cm <sup>2</sup> /168hrs)		
	Maximum allowed	As received	10 %
Magnesium (AZ 31B-H24) AMS 4377 surface treatment per SAE AMS-M-3171, Ty III	0.50	0.07	<b>NOT PERFORMED</b>
Aluminum, SAE AMS-QQ-A-250/4, T3 surface treatment per MIL-A-8625, Type I, Class I	0.15	0.02	
Aluminum, SAE AMS-QQ-A-250/4, Bare T3 Alloy	0.15	0.02	
Aluminum, SAE AMS-QQ-A-250/12, Bare T6 Alloy	0.15	0.01	
Titanium, SAE AMS-T-9046, 6Al-4V CI III, Comp. C	0.10	0.01	
Steel, AMS 5046, Grade 1020	0.25	0.02*	
Steel, 410 SS, Silver Plated per SAE AMS 2410	0.10	0.02	

\*Slight discoloration

Result Conforms\*

- 3.7.3 **Low-embrittling cadmium plate corrosion:** Steel panels coated with low-embrittling cadmium plate immersed in the concentrated cleaning compound (all types) and a 10% solution of the cleaning compound (Types I, II and IV only) in distilled water shall not show a weight change greater than 0.14 mg/cm<sup>2</sup> for 24 hours when tested in accordance with 4.5.11.

**As received:** 0.07\* mg/cm<sup>2</sup>/24hrs

**Dilute (10 %):** Not performed

Result Conforms\*

\* Test performed using "as received" solution (ready to use) instead of dilution required by specification.

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- 3.7.4 Effects on unpainted metal surfaces: The concentrated cleaning compound (Type III only) and a 10% solution (Types I, II and IV only) of the cleaning compound in distilled water shall not cause streaking, stains or other deposits that cannot be easily removed with water when tested in accordance with 4.5.12.

Result Conforms\*

- 3.7.5 Sandwich corrosion: When tested in accordance with 4.5.16, the concentrated cleaner (all types) and a 10% solution (Types I, II and IV only) shall show no corrosion in excess of that shown by control test coupons in ASTM D 1193, Type IV, reagent water.

	2024-T3 Bare Anodized	2024-T3 Alclad	7075-T6 Bare Anodized	7075-T6 Alclad
As received	1	1	1	1
Dilute (10%)	Not performed			
Control	1	1	1	1

<sup>1</sup>staining/etching in excess of 25% of the surface area

Result Conforms\*

- 3.7.6 Wet adhesion tape test (Types II and IV): A ten (10) percent solution of the cleaning compound, when used as directed, shall remove soil from a painted surface in preparation for repainting such that paint applied after cleaning with the compound shall adhere to the surface when tested in accordance with 4.5.27.

COATING SYSTEM	OBSERVATIONS
<b>SET 1:</b> Primer: MIL-PRF-85582, Type I, Class 1B Waterborne Epoxy Topcoat: MIL-PRF-85285 Type I High Solids Polyurethane, Color # 34092	Coating system showed no sign of damage.
<b>SET 2:</b> Primer: MIL-PRF-23377, Type I, Class C High Solids Epoxy Topcoat: MIL-PRF-85285 Type I High Solids Polyurethane, Color # 34092	Coating system showed no sign of damage.
<b>SET 3:</b> Primer: TT-P-2760, Type I, Class C High Solids Elastomeric, Polyurethane Topcoat: MIL-C-85285 Type I High Solids Polyurethane, Color # 34092	Coating system showed no sign of damage.

Result Conforms\*

\* Test performed using "as received" solution (ready to use) instead of dilution required by specification.

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- 3.8 Effect on painted surfaces: The concentrated cleaning compound (Type III only) and a 25% solution (Types I, II and IV) of the cleaning compound in distilled water shall not cause streaking, blistering, discoloration or a permanent decrease in film hardness of more than one (1) pencil hardness level when tested in accordance with 4.5.13. The Type I material shall be tested using only the (H) Polyurethane paint systems.

PANEL SET	RESULT	
	Conc.	25 %
<b>E (Epoxy topcoat)</b> Primer: MIL-PRF-23377, Ty I, Class C High-Solids Epoxy Primer Topcoat: MIL-PRF-22750 Epoxy Topcoat, Color #: 17925	Pass	Not performed
<b>H (Polyurethane)</b> Primer: MIL-PRF-23377, Ty I, Class C High-Solids Epoxy Primer Topcoat: MIL-PRF-85285 Ty I, Polyurethane, High Solids, Color #: 17925	Pass	
<b>F (Enamel)</b> Primer: MIL-PRF-23377, Ty I, Class C High-Solids Epoxy Primer Topcoat: TT-E-529 Enamel, Semi-gloss, Color #: 27925	Pass	

Result Conforms\*

- 3.9 Stress crazing of MIL-PRF-5425 and MIL-PRF-25690 (Type A and C) acrylic plastics: The concentrated product (Type III only) and a 10% solution (Types I, II and IV) in distilled water shall not cause stress crazing or staining of acrylic plastics when tested in accordance with 4.5.14.

Material	As received	Dilution (10%)
MIL-PRF-5425 (Type A)	<b>*Crazing: Fails</b>	Not performed
MIL-PRF-25690 (Type C)	<b>No crazing: Pass</b>	Not performed

Result \*Does not conform

\* Test performed using "as received" solution (ready to use) instead of dilution required by specification.

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- 3.10 Stress crazing of polycarbonate plastic: The concentrated product (Type III only) and a 10% solution (Types I, II and IV) in distilled water shall not cause stress crazing or staining of polycarbonate plastic conforming to MIL-P-83310 when tested in accordance with 4.5.15.

Material	As received	Dilution (10%)
MIL-P-83310 (Polycarbonate)	<b>Crazing: Fails</b>	Not performed

Result \*Does not conform

- 3.11 Long-term storage stability: After being stored for a period of 12 months, in accordance with 4.5.17, the cleaning compound shall not layer, separate, precipitate or corrode the shipping container. Plastic containers shall not show leakage nor any cracking, crazing, or softening. All cleaning compounds shall meet the requirements of paragraphs 3.5.1, 3.7.1, 3.7.2, 3.15, and 3.16 of this specification.

Result Not performed

- 3.12 Hot dip galvanizing corrosion: The concentrated product (Type III only) and a 10% solution of the cleaning compound (Types I, II and IV) in distilled water shall not show a weight change of an average of three (3) test panels greater than 0.14 mg/cm<sup>2</sup> when tested in accordance with 4.5.18.

**As received:** 0.04 mg/cm<sup>2</sup>  
**Dilute (10%):** Not performed

Result \*Conforms

- 3.13 Workmanship: The cleaning compound shall be a liquid having a uniform and homogenous appearance. The cleaning compound shall be manufactured from materials that shall produce a product harmless to metal surfaces and humans when used as directed.

Result To be Cert. by Mfr.

*\* Test performed using "as received" solution (ready to use) instead of dilution required by specification.*

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- 3.14 Effect on polysulfide sealants: The concentrated cleaning compound (Type III only) and a 25% solution (Types I, II and IV) of the cleaning compound in distilled water shall not change the durometer hardness of the polysulfide sealant by more than 5 units when tested in accordance with 4.5.19.

**Sealants:**    **MIL-S-81733 Type 1:**    **< 5 units hardness change**  
                  **MIL-S-8802 Type 1:**    **< 5 units hardness change**

Result \_\_\_\_\_ Conforms\*

- 3.15 Rubber compatibility: The concentrated cleaning compound (Type III only) and a 25% solution (Types I, II and IV only) of the cleaning compound in distilled water shall not change the durometer hardness more than 5 units when tested in accordance with 4.5.20.

**Rubbers:**    **AMS 3204:**    **< 5 units hardness change**  
                  **AMS 3209:**    **< 5 units hardness change**

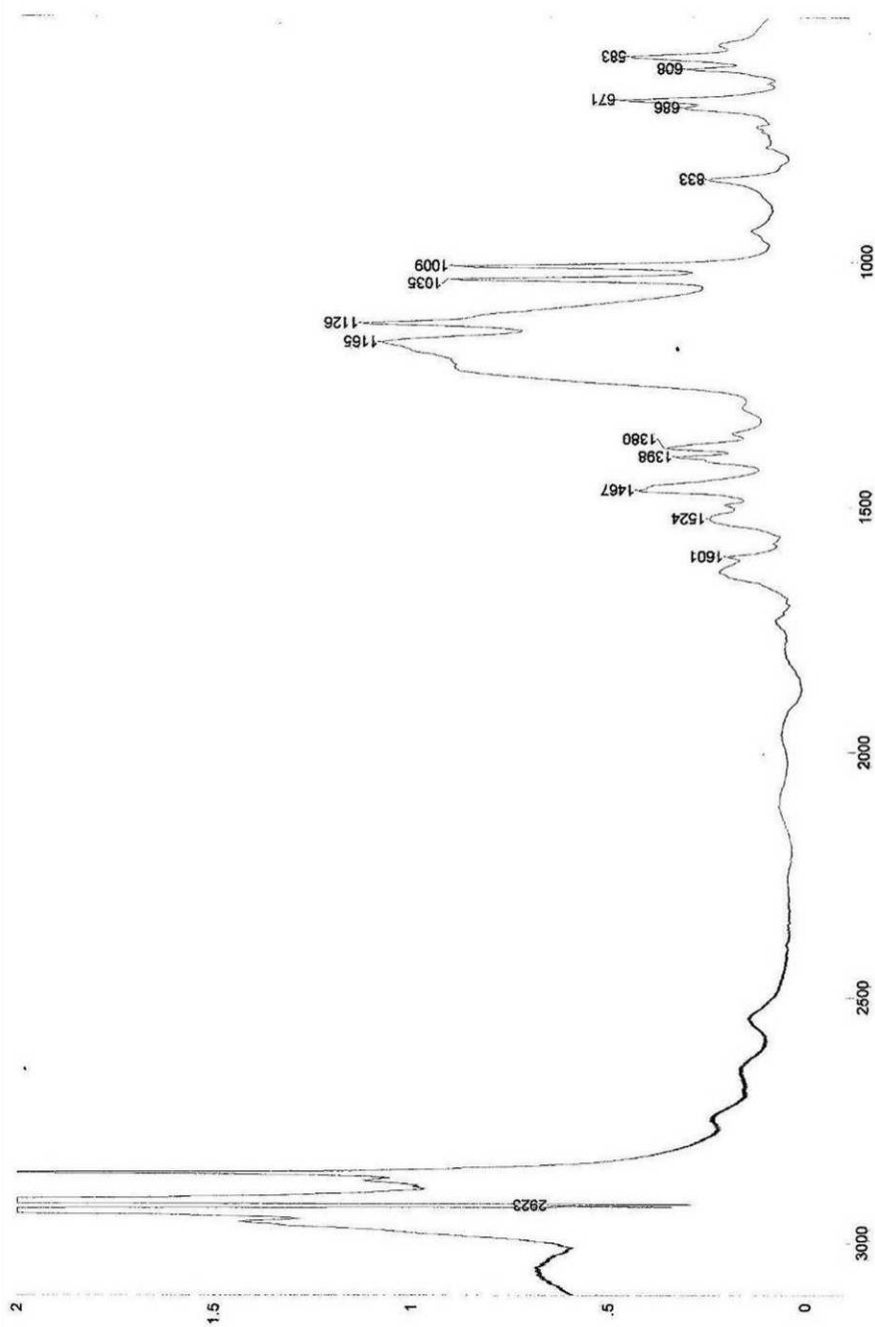
Result \_\_\_\_\_ Conforms\*

- 3.16 Effect on polyimide insulated wire: The cleaning compound, when tested according to 4.5.26, shall not cause dissolution, cracking, or dielectric breakdown (leakage) of the polyimide insulated wire in excess of that produced by distilled water.

**Polyimide wire did not exhibit dissolution, cracking or dielectric breakdown.**

Result \_\_\_\_\_ Conforms

\* Test performed using "as received" solution (ready to use) instead of dilution required by specification.



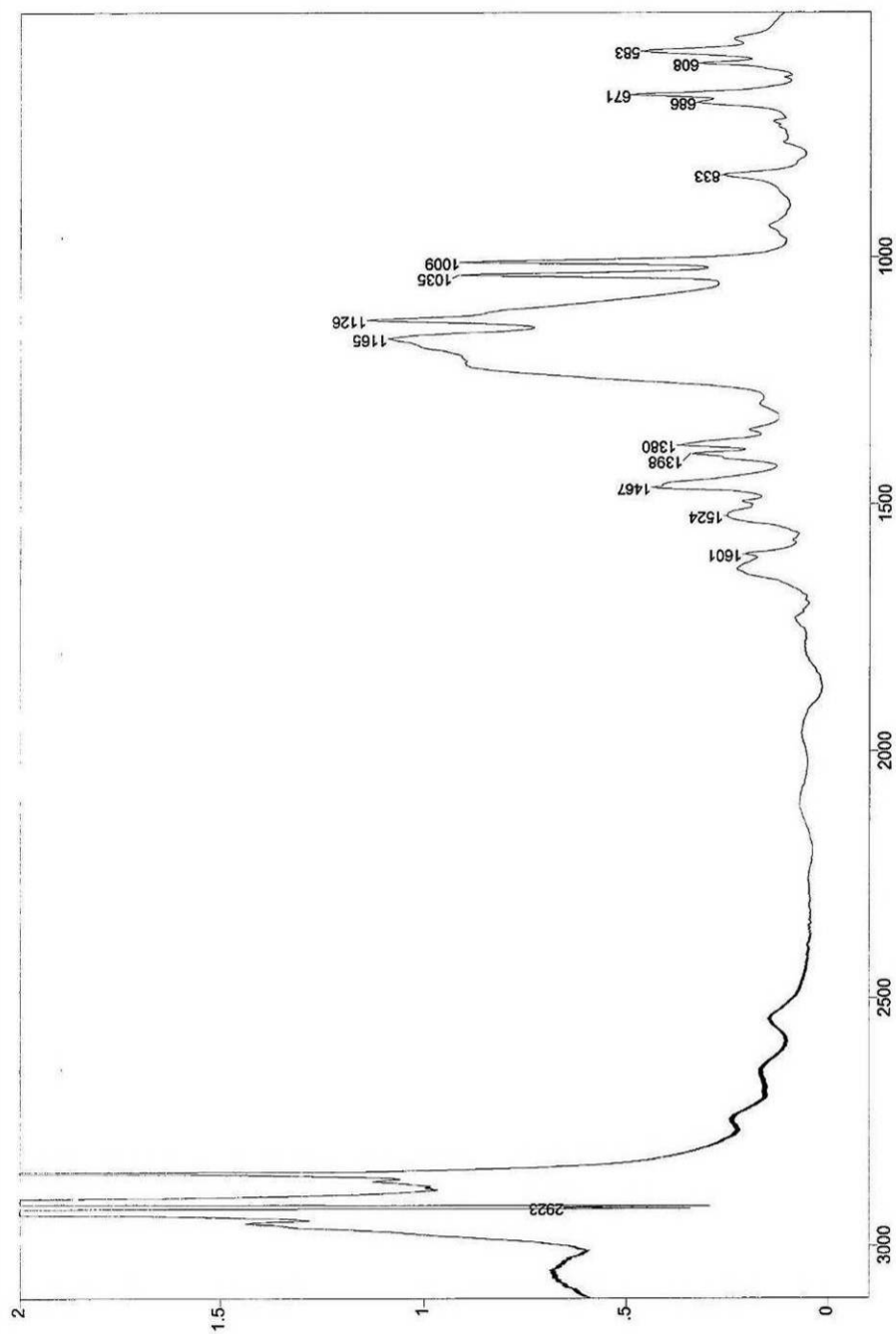
Absorbance / Wavenumber (cm-1)

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4dec086 BATTLE EAGLE KLEEN MIL-PRF-87937D TY IV

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Absorbance / Wavenumber (cm-1)

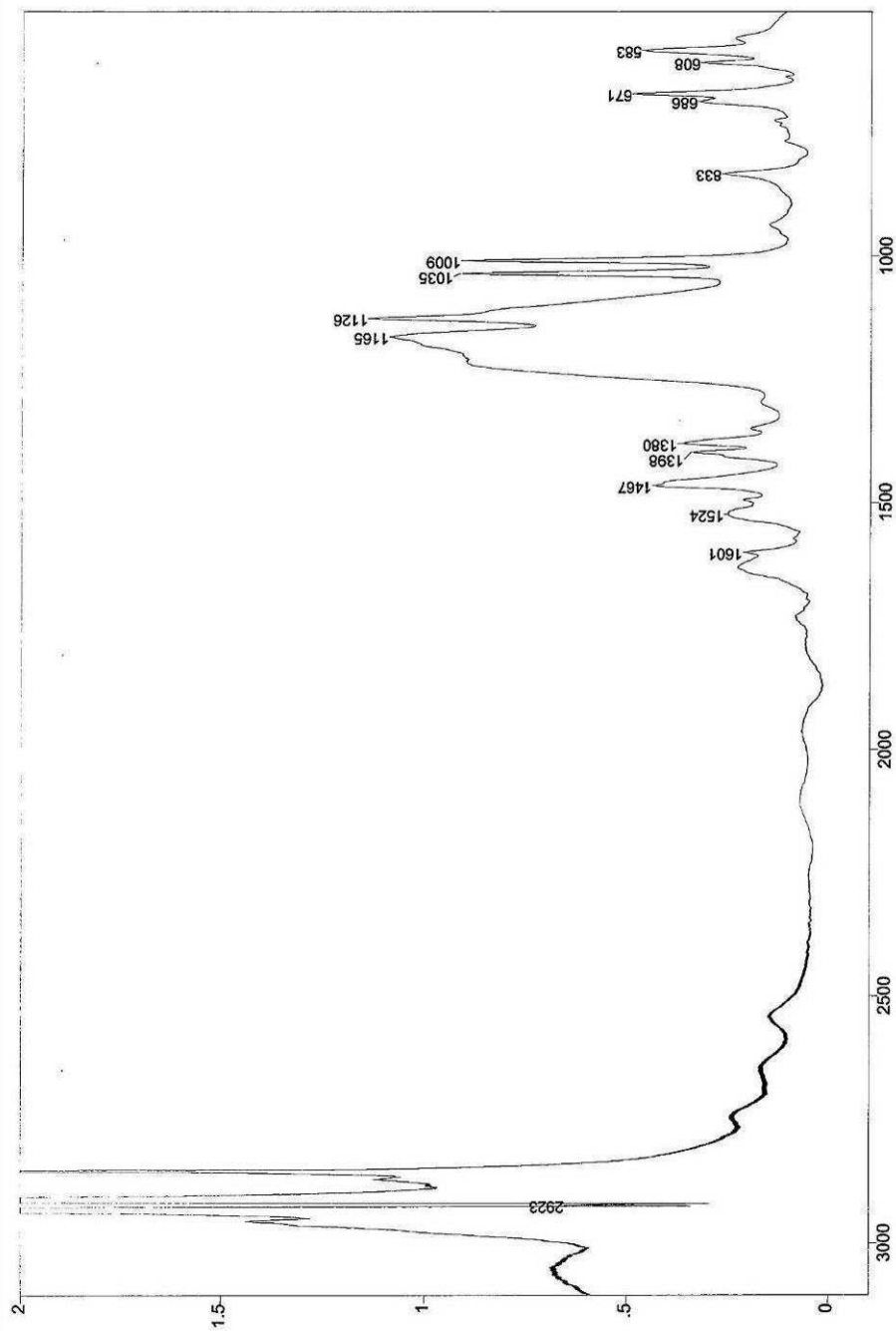
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4dec086 BATTLE EAGLE KLEEN MIL-PRF-87937D TY IV

Paged Z-Zoom CURSOR

11-02-2005 3:51 PM Res=2 cm-1





Absorbance / Wavenumber (cm-1)

Paged Z-Zoom CURSOR

File # 4 : 5FEB248B

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4dec086 BATTLE EAGLE KLEEN MIL-PRF-87937D TY IV

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Attn: Sara Kuczek  
Battelle Memorial Institute  
505 King Avenue  
Columbus, OH 43201-2693

Date: 04-Mar-2005

SMI/REF: 04DEC086

Product: **Eagle Kleen III Lot # C531-84-1** (received 01-December-2004)

Dilution: Ready to use

ASTM D 323  
Test Method for Vapor Pressure of  
Petroleum Products (Reid Method)

## SCOPE

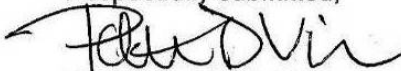
This test method provides procedures for the determination of vapor pressure of gasoline, volatile crude oil, and other volatile petroleum products.

## SUMMARY OF TEST METHOD

The liquid chamber of the vapor pressure apparatus is filled with the chilled sample and connected to the vapor chamber that has been heated to 37.8°C (100°F) in a bath. The assembled apparatus is immersed in a bath at 37.8°C (100°F) until a constant pressure is observed. The reading, suitably corrected, is reported as the Reid vapor pressure.

Sample	Reid Vapor Pressure
Eagle Kleen III	<0.2 mm Hg @ 20°C

Respectfully submitted,



Patricia D. Viani, SMI Inc.

# SMI, Inc.

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Attn: BATTELLE MEMORIAL INSTITUTE  
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Date: 07-Mar-2005  
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PRODUCT: **EAGLE KLEEN III** (Lot # C531-84-1)  
(received 01-Dec-2004)

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## 40 CFR 796.3100: AEROBIC AQUATIC BIODEGRADATION

Code of Federal Regulations  
Environmental Protection Agency  
Title 40: Protection of Environment  
Part 796: Chemical Fate Testing Guidelines  
Shake Flask Method

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### Summary of Results:

Based on dissolved organic carbon analysis:

<p><b>"EAGLE KLEEN III" = 87.1 % Biodegradable in 28 days</b></p>
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See Appendix A for graphical representation of Biodegradability vs. Time .

### PROCEDURE

#### I. Introduction

This procedure provides a way to determine the rate and extent of aerobic biodegradation that might occur when chemical substances are released to aquatic environments. A high biodegradability result in this test provides evidence that the test substance will be biodegradable in natural aerobic freshwater environments. A low biodegradability result may not necessarily indicate poor biodegradation, as other factors may interfere, such as inhibition of the microbial inoculum by the test material.

Client: Battelle Memorial Institute  
Product: EAGLE KLEEN III

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## **EPA 796.3100: AEROBIC AQUATIC BIODEGRADATION**

### **II. Principle of the Test Method**

The method consists of a 2-week inoculum buildup period during which the microbes are allowed to adapt to the test compound. The acclimated media containing a defined amount of test compound is added to specially equipped Erlenmeyer flasks. The test media is sampled periodically and analyzed for dissolved organic carbon (DOC). A reservoir filled with barium hydroxide is utilized to measure the amount of carbon dioxide evolved. The degree of biodegradation is determined by comparison of the extent of DOC disappearance and the amount of carbon dioxide liberated. Control flasks containing no test compounds are run simultaneously and are used to estimate the degree of ultimate biodegradation. Reference substances which will exhibit ultimate biodegradation may be run simultaneously to check the activity of the inoculum. If the reference samples do not exhibit at least 60 percent of theoretical maximum carbon dioxide, and at least 70 percent DOC removal within 28 days, the test will be regarded as invalid and shall be repeated using different inoculum.

This method is believed to be appropriate for a screening test which has solely an acceptance but no rejective function.

### **III. Test Procedure**

The total organic carbon (TOC) of the test compound is first determined by analysis or calculation if the formulation is known. Determination of the minimum inhibitory concentration is useful to insure that the test compound will not be inhibitory to the microbes at the required concentration. The shake flask apparatus is assembled utilizing a 2-liter Erlenmeyer flask and a 50 ml centrifuge tube. The tube containing 10 mls of barium hydroxide will be suspended over the contents of the flask in such a way that liberated carbon dioxide may diffuse into the barium hydroxide, while allowing the contents of the tube to be removed for analysis without spilling into the test media. Glass tubing may be utilized as access into the flask for sparging, venting, and sampling.

Stock solutions I, II, and III are prepared (see Appendix B), along with 0.2 N barium hydroxide and 0.1 N HCl. Acclimation medium is prepared by adding 1 ml each of stock solutions I, II and III to 1 liter of distilled, deionized water (DIW). The microbial inoculum is obtained from sewage and soil or from Polyseed and is added to the acclimation medium. Test compounds are added incrementally during the acclimation period at concentrations equivalent to 4, 8, and 8 mg/L carbon on days 0, 7, and 11, respectively. On day 14, the medium is ready for use in the test.

Client: Battelle Memorial Institute  
Product: EAGLE KLEEN III

Date: 07-Mar-2005  
SMI REF: 04DEC086  
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#### **EPA 796.3100: AEROBIC AQUATIC BIODEGRADATION**

Biodegradability test flasks are prepared by adding 100 mls of acclimation medium to 900 mls of DIW along with 1 ml each of solutions I, II, and III to the 2-liter Erlenmeyers. Additional test compound equivalent to 10 mg/L carbon is added to the flasks. Ten mls of barium hydroxide are added to the suspended reservoirs in each flask and 10 mls are also saved for use as a titration blank. Flasks are sparged with carbon dioxide-free air, sealed and placed on a shaking table (approx. 125 rpm) at 20 - 25 deg C in the dark. Test flasks should be run in triplicate and sampling should occur at time zero and at least four other times to allow for a smooth plot of biodegradation. Each sample for DOC analysis is first centrifuged or filtered through a 0.45 micrometer or smaller pore diameter. On the day prior to terminating the test, 3 mls of 20 percent sulfuric acid are added to release carbonate bound carbon dioxide.

#### **IV. ANALYTICAL MEASUREMENTS**

The quantity of carbon dioxide evolved is measured by titration of the entire barium hydroxide sample with 0.1 N HCl to the phenolphthalein end point, blank subtracted. Theoretically, 10 mg of carbon is converted to 0.833 mmol of carbon dioxide. Absorbed carbon dioxide precipitates as barium carbonate, causing a reduction in alkalinity by the equivalent of 16.67 ml of 0.1 N HCl for complete conversion of the test compound carbon to carbon dioxide. Therefore, the percent theoretical carbon dioxide evolved from the test compound is calculated at any sampling time from the formula:

$$\% \text{ CO}_2 \text{ evolution} = [(TF - CF)/16.67] \cdot 100$$

where:

TF = mls of 0.1 N HCl used in titration of test flask

CF = mls of 0.1 N HCl used in titration of control flask

Client: Battelle Memorial Institute  
Product: EAGLE KLEEN III

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**EPA 796.3100: AEROBIC AQUATIC BIODEGRADATION**

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The DOC analysis is performed using a suitable organic carbon method. The percent DOC disappearance from the test compound is calculated from the formula:

$$\% \text{ DOC removal} = [1 - (\text{DTF}_x - \text{DCF}_x) / (\text{DTF}_o - \text{DCF}_o)] \cdot 100$$

where:

DTF = Dissolved organic carbon from test flask  
DCF = Dissolved organic carbon from control flask

o = Day zero measurements  
x = Day x measurements

**V. REPORT OF RESULTS**

Inoculum: Polyseed and Mixed inoculum

Date Received: Jan, 2004

Source: Fisher Scientific and Metro-Dade County Water & Sewer Authority

Storage: Ambient temperature, used within 24 hours

Minimum Inhibitory Concentration: MIC < 3.125 % (non-inhibitory to microbes at concentrations lower than 3.125%)

Percent Biodegradation based on DOC analysis:

<b>EAGLE KLEEN III:</b>	87.1 % after 28 days (see Table 1)
Reference (Sodium citrate):	92.6 % after 28 days (see Table 1)

Client: Battelle Memorial Institute  
Product: EAGLE KLEEN III

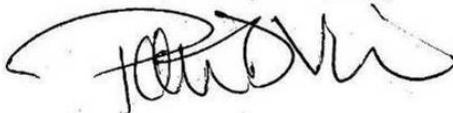
Date: 07-Mar-2005  
SMI REF: 04DEC086  
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**EPA 796.3100: AEROBIC AQUATIC BIODEGRADATION**

Summary: Since the test compound was found to be over 70 % biodegradable based on the DOC analysis, it is reasonable to assume that the substance will undergo rapid and ultimate biodegradation in aerobic aquatic environments, also known as "ready biodegradability". The test is validated by the fact that the reference compound, sodium citrate, exhibited a biodegradability over 70%.

The percent biodegradability based on carbon dioxide evolution is typically lower than that of the DOC based numbers. In this case, the carbon dioxide evolution measured was significant, both on the test compound and on the reference, and the results generally agree.

Respectfully submitted,

A handwritten signature in black ink, appearing to read 'Patricia D. Viani', with a large, sweeping flourish at the end.

Patricia D. Viani  
SMI, Inc.

Client: Battelle Memorial Institute  
Product: EAGLE KLEEN III

Date: 07-Mar-2005  
SMI REF: 04DEC086  
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**EPA 796.3100: AEROBIC AQUATIC BIODEGRADATION**

TABLE I - DISSOLVED ORGANIC CARBON (DOC) VALUES

Sample: **EAGLE KLEEN III**

	DAY 0	DAY 7	DAY 14	DAY 21	DAY 28
A	35.7	11.6	9.2	8.3	7.3
B	39.1	9.3	8.8	7.2	7.4
C	37.0	15.2	13.8	9.9	8.5
AVERAGE	37.3	12.0	10.6	8.5	7.7
CORRECTED AV	34.0	8.7	7.2	5.1	4.4
% BIODEGRADED	N/A	74.3%	78.7%	85.1%	87.1%

Reference: Sodium Citrate

A	38.0	14.2	6.8	5.2	5.5
B	39.9	9.1	6.9	6.2	6.0
C	39.1	8.4	7.3	6.7	6.5
AVERAGE	39.0	10.6	7.0	6.0	6.0
CORRECTED AV	35.7	7.3	3.6	2.6	2.6
% BIODEGRADED	N/A	79.6%	89.8%	92.6%	92.6%

BLANK	A	3.5	3.1	3.3	3.7	3.0
	B	3.0	3.6	3.6	3.2	3.7
	C	3.4	3.2	3.2	3.3	3.4
AVERAGE		3.3	3.3	3.4	3.4	3.4



Client: Battelle Memorial Institute  
Product: EAGLE KLEEN III

Date: 07-Mar-2005  
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**EPA 796.3100: AEROBIC AQUATIC BIODEGRADATION**

Table II - Titration Data for CO2 Evolution

Sample: **EAGLE KLEEN III**

		DAY 7	DAY 14	DAY 21	DAY 28
	A	6.8	8.8	15.8	18.0
	B	5.6	12.4	16.0	18.0
	C	6.0	9.2	16.6	17.6
AVERAGE		6.1	10.1	16.1	17.9
CORRECTED AVG		11.9	7.6	1.9	0.2
% BIODEGRADED		20.9%	13.4%	3.3%	0.4%
mls theoretical:	56.7			<b>TOTAL=</b>	<b>38.0%</b>

Reference: Sodium Citrate

	A	4.6	7.8	17.0	18.0
	B	6.0	9.2	14.8	17.8
	C	5.2	6.2	16.8	18.0
AVERAGE		5.3	7.7	16.2	17.9
CORRECTED AVG		12.7	10.0	1.8	0.1
% BIODEGRADED		21.4%	16.8%	3.0%	0.2%
mls theoretical:	59.5			<b>TOTAL =</b>	<b>41.4%</b>

BLANK	A	18.2	18.0	18.2	17.8
	B	18.2	17.4	18.0	17.8
	C	17.6	17.8	17.8	18.6

AVERAGE		18.0	17.7	18.0	18.1
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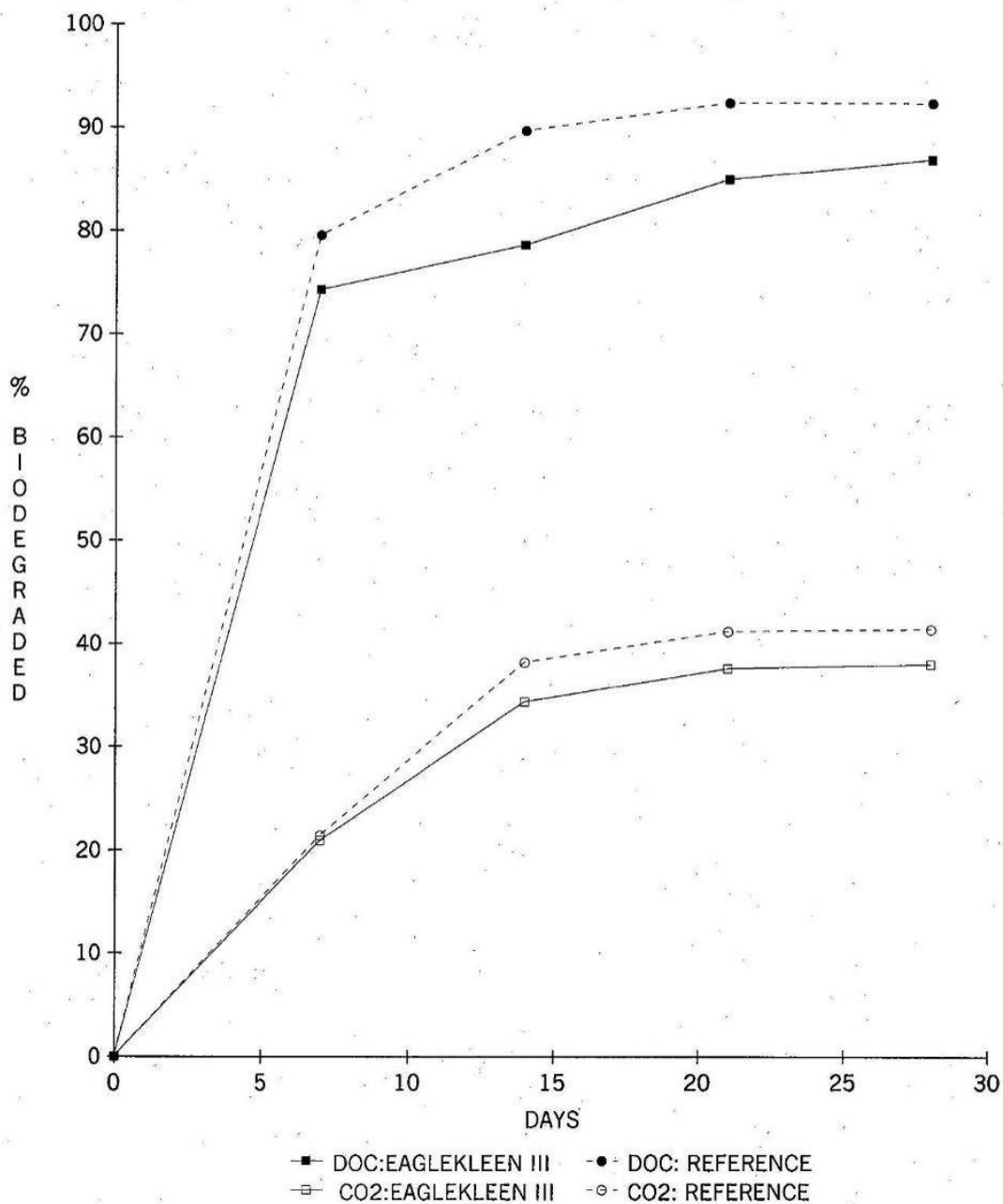
Client: Battelle Memorial Institute  
Product: EAGLE KLEEN III

Date: 07-Mar-2005  
SMI REF: 04DEC086  
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**EPA 796.3100: AEROBIC AQUATIC BIODEGRADATION**

Appendix A

**BIODEGRADABILITY VS. TIME**  
EAGLE KLEEN III



Date: 07-Mar-2005  
SMI REF: 04DEC086  
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## Appendix B

### STOCK SOLUTIONS I, II, AND III

SOLUTION II:	25	g/L	$\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$
	10	g/L	KCl
	20	g/L	$\text{MgSO}_4$
	1	g/L	$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$

adjust pH of Soln II to 3.0

SOLUTION III:	5	g/L	CaCl <sub>2</sub>
	0.05	g/L	ZnCl <sub>2</sub>
	0.5	g/L	MnCl <sub>2</sub> ·4H <sub>2</sub> O
	0.05	g/L	CuCl <sub>2</sub>
	0.001	g/L	CoCl <sub>2</sub>
	0.001	g/L	H <sub>3</sub> BO <sub>3</sub>
	0.0004	g/L	MoO <sub>3</sub>

# SMI, Inc.

12219 SW 131 Avenue  
Miami, Florida 33186-6401 USA

Phone: (305) 971-7047  
Fax: (305) 971-7048

Attn: Nick Conkle  
Battelle Memorial Institute  
505 King Ave  
Columbus, OH 43201

Date: 24-Jun-2005

SMI/REF: 04JUN604

Product: **EAGLE KLEEN III**  
(received 20-Jun-2005)

Dilution: Ready to use

Page 1 of 1

Modified testing (product was tested neat - [undiluted])

**MIL-PRF-87937D** (24 Sep 2001)

CLEANING COMPOUND, AEROSPACE EQUIPMENT

Type IV - Heavy Duty, Water Dilutable Cleaning Compound

- 3.9 Stress crazing of MIL-PRF-5425 and MIL-PRF-25690 (Type A and C) acrylic plastics: The concentrated product (Type III only) and a 10% solution (Types I, II and IV) in distilled water shall not cause stress crazing or staining of acrylic plastics when tested in accordance with 4.5.14.

Material	As received	Dilution (10%)
MIL-PRF-5425 (Type A)	<b>Crazing: Fails</b>	Not performed
MIL-PRF-25690 (Type C)	<b>No crazing: Pass</b>	Not performed

Result Does not conform

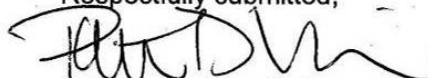
- 3.10 Stress crazing of polycarbonate plastic: The concentrated product (Type III only) and a 10% solution (Types I, II and IV) in distilled water shall not cause stress crazing or staining of polycarbonate plastic conforming to MIL-P-83310 when tested in accordance with 4.5.15.

Material	As received	Dilution (10%)
MIL-P-83310 (Polycarbonate)	<b>Crazing: Fails</b>	Not performed

Result Does not conform

\* Tests performed using "as received" solution (ready to use) instead of dilution required by specification.

Respectfully submitted,



Patricia D. Viani, SMI Inc.

## **Appendix E**

### **SMI Results from Eagle Kleen III Vapor Pressure Testing**

# SMI, Inc.

12219 SW 131 Avenue  
Miami, Florida 33186-6401 USA

Phone: (305) 971-7047  
Fax: (305) 971-7048

Attn: Sara Kuczek  
Battelle Memorial Institute  
505 King Avenue  
Columbus, OH 43201-2693

Date: 04-Mar-2005

SMI/REF: 04DEC086

Product: **Eagle Kleen III Lot # C531-84-1** (received 01-December-2004)

Dilution: Ready to use

ASTM D 323  
Test Method for Vapor Pressure of  
Petroleum Products (Reid Method)

## SCOPE

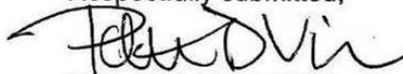
This test method provides procedures for the determination of vapor pressure of gasoline, volatile crude oil, and other volatile petroleum products.

## SUMMARY OF TEST METHOD

The liquid chamber of the vapor pressure apparatus is filled with the chilled sample and connected to the vapor chamber that has been heated to 37.8°C (100°F) in a bath. The assembled apparatus is immersed in a bath at 37.8°C (100°F) until a constant pressure is observed. The reading, suitably corrected, is reported as the Reid vapor pressure.

Sample	Reid Vapor Pressure
Eagle Kleen III	<0.2 mm Hg @ 20°C

Respectfully submitted,



Patricia D. Viani, SMI Inc.

## **Appendix F**

### **SMI Results from Eagle Kleen III Toxicity Testing**

# SMI, Inc.

12219 SW 131 Avenue  
Miami, Florida 33186-6401 USA

Phone: (305) 971-7047  
Fax: (305) 971-7048

Attn: Nick Conkle  
Battelle Memorial Institute  
505 King Avenue  
Columbus, Oh 43201-2693

Date: 09-Dec-2005

SMI/REF: 05XCT045

Product: **EAGLE KLEEN III (50540-98-5)** (received 27-Oct-2005)

Dilution: Ready to Use

Page 1 of 1

Partial testing in accordance with:  
**AMS 1424F**  
Deicing/Anti-icing, Fluid, Aircraft SAE Type I  
(Fluid is ready to use)

3.1.4.4 **Aquatic Toxicity:** Formulated fluid shall be tested in accordance with EPA (40CFR 797.1300 and 797.1400, revised July 1, 1989 and 40 CFR 136.3) or OECD (Organization for Economic Cooperation and Development Guidelines for Testing of Chemicals, Methods 202 and 203) procedures using test species required by regulatory agencies for permitted discharges. Examples include: fathead minnows (96-hour  $LC_{50}$ ), *Ceriodaphnia dubia* (48-hour  $EC_{50}$ ), *Daphnia magna* (48-hour  $EC_{50}$ ) and rainbow trout (96-hour  $LC_{50}$ ). The  $LC_{50}$  (for fish) or  $EC_{50}$  (for invertebrates) concentration (the highest concentration at which 50% of the organisms do not survive the test period) shall be given in milligrams per liter.

## EPA 40 CFR 797.1300 DAPHNID ACUTE TOXICITY TEST

*Daphnia magna*, static system

**48 hour  $LC_{50}$ : 25 mg/L**

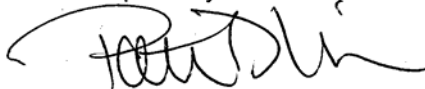
## EPA 40 CFR 797.1400 FISH ACUTE TOXICITY TEST

*Pimephales promelas*, static system

**96 hour  $LC_{50}$ : 30 mg/L**

Result Informational

Respectfully submitted,



Patricia D. Viani, SMI, Inc.



# SMI, Inc.

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Attn: Nick Conkle  
Battelle Memorial Institute  
505 King Avenue  
Columbus, Oh 43201-2693

Date: 09-Dec-2005

SMI/REF: 05XCT046

Product: **SAFETY KLEEN (50540-98-24)** (received 27-Oct-2005)

Dilution: Ready to Use

Page 1 of 1

Partial testing in accordance with:

**AMS 1424F**

Deicing/Anti-icing, Fluid, Aircraft SAE Type I  
(Fluid is ready to use)

3.1.4.4 **Aquatic Toxicity:** Formulated fluid shall be tested in accordance with EPA (40CFR 797.1300 and 797.1400, revised July 1, 1989 and 40 CFR 136.3) or OECD (Organization for Economic Cooperation and Development Guidelines for Testing of Chemicals, Methods 202 and 203) procedures using test species required by regulatory agencies for permitted discharges. Examples include: fathead minnows (96-hour  $LC_{50}$ ), *Ceriodaphnia dubia* (48-hour  $EC_{50}$ ), *Daphnia magna* (48-hour  $EC_{50}$ ) and rainbow trout (96-hour  $LC_{50}$ ). The  $LC_{50}$  (for fish) or  $EC_{50}$  (for invertebrates) concentration (the highest concentration at which 50% of the organisms do not survive the test period) shall be given in milligrams per liter.

EPA 40 CFR 797.1300 DAPHNID ACUTE TOXICITY TEST

*Daphnia magna*, static system

48 hour  $LC_{50}$ : 125 mg/L

EPA 40 CFR 797.1400 FISH ACUTE TOXICITY TEST

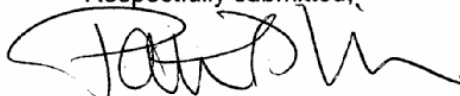
*Pimephales promelas*, static system

96 hour  $LC_{50}$ : >70,000 mg/L\*

**\*Note: Product left a distinct layer on top of water layer. Since product was not water soluble, results are suspect.**

Result Informational

Respectfully submitted,



Patricia D. Viani, SMI, Inc.

SCIENTIFIC MATERIAL INTERNATIONAL  
www.smiinc.com

# SMI, Inc.

12219 SW 131 Avenue  
Miami, Florida 33186-6401 USA

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Fax: (305) 971-7048

Attn: Nick Conkle  
Battelle Memorial Institute  
505 King Avenue  
Columbus, Oh 43201-2693  
Date: 09-Dec-2005  
SMI/REF: 05XCT047

Product: **TURCO 6849 SOLN 20% by volume (50540-98-13)** (received 27-Oct-2005)

Dilution: Ready to Use  
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Partial testing in accordance with:

**AMS 1424F**

Deicing/Anti-icing, Fluid, Aircraft SAE Type I  
(Fluid is ready to use)

3.1.4.4 **Aquatic Toxicity:** Formulated fluid shall be tested in accordance with EPA (40CFR 797.1300 and 797.1400, revised July 1, 1989 and 40 CFR 136.3) or OECD (Organization for Economic Cooperation and Development Guidelines for Testing of Chemicals, Methods 202 and 203) procedures using test species required by regulatory agencies for permitted discharges. Examples include: fathead minnows (96-hour  $LC_{50}$ ), *Ceriodaphnia dubia* (48-hour  $EC_{50}$ ), *Daphnia magna* (48-hour  $EC_{50}$ ) and rainbow trout (96-hour  $LC_{50}$ ). The  $LC_{50}$  (for fish) or  $EC_{50}$  (for invertebrates) concentration (the highest concentration at which 50% of the organisms do not survive the test period) shall be given in milligrams per liter.

EPA 40 CFR 797.1300 DAPHNID ACUTE TOXICITY TEST

*Daphnia magna*, static system

**48 hour  $LC_{50}$ : 150 mg/L**

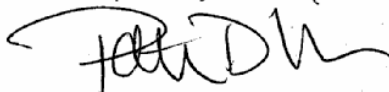
EPA 40 CFR 797.1400 FISH ACUTE TOXICITY TEST

*Pimephales promelas*, static system

**96 hour  $LC_{50}$ : 225 mg/L**

Result Informational

Respectfully submitted,



Patricia D. Viani, SMI, Inc.

# SMI, Inc.

12219 SW 131 Avenue  
Miami, Florida 33186-6401 USA

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Fax: (305) 971-7048

Attn: Nick Conkle  
Battelle Memorial Institute  
505 King Avenue  
Columbus, Oh 43201-2693

Date: 09-Dec-2005

SMI/REF: 05XCT048

Product: **RUST BLOC SOLN 4% by weight (50540-98-20)** (received 27-Oct-2005)

Dilution: Ready to Use

Page 1 of 1

Partial testing in accordance with:

**AMS 1424F**

Deicing/Anti-icing, Fluid, Aircraft SAE Type I  
(Fluid is ready to use)

3.1.4.4 Aquatic Toxicity: Formulated fluid shall be tested in accordance with EPA (40CFR 797.1300 and 797.1400, revised July 1, 1989 and 40 CFR 136.3) or OECD (Organization for Economic Cooperation and Development Guidelines for Testing of Chemicals, Methods 202 and 203) procedures using test species required by regulatory agencies for permitted discharges. Examples include: fathead minnows (96-hour  $LC_{50}$ ), *Ceriodaphnia dubia* (48-hour  $EC_{50}$ ), *Daphnia magna* (48-hour  $EC_{50}$ ) and rainbow trout (96-hour  $LC_{50}$ ). The  $LC_{50}$  (for fish) or  $EC_{50}$  (for invertebrates) concentration (the highest concentration at which 50% of the organisms do not survive the test period) shall be given in milligrams per liter.

EPA 40 CFR 797.1300 DAPHNID ACUTE TOXICITY TEST

*Daphnia magna*, static system

**48 hour  $LC_{50}$ : 79,200 mg/L**

EPA 40 CFR 797.1400 FISH ACUTE TOXICITY TEST

*Pimephales promelas*, static system

**96 hour  $LC_{50}$ : 33,500 mg/L**

Result Informational

Respectfully submitted,



Patricia D. Viani, SMI, Inc.